A TURBIDIMETRIC METHOD FOR ESTIMATING THE NUMBER OF NEMATODE LARVAE IN A SUSPENSION.

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(Two Text-figures.)

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Synopsis.

A turbidimetric method for estimating the number of nematode larvae in an aqueous suspension stabilized with 0·5% carboxymethylcellulose is described. A simply constructed, modified Peter's counting chamber is used to count the nematode larvae in a number of 10 ml. standardizing suspensions. The light absorption due to the turbidity of each of these suspensions is measured with a Hilger "Spekker" absorptiometer. A standard curve relating the logarithm of absorption and the number of nematodes in the suspension is constructed and used subsequently to predict the number of nematodes in a suspension, the logarithm of absorption of which has been measured.

The results are rapidly obtained, amenable to statistical analysis and for *Anguina agrostis* (Steinbuch) Goodey, the percentage error in the prediction from the standard curve was less than 5%.

Introduction.

During the course of an investigation into the aetiology of the plant parasitic nematode *Anguina agrostis* (Steinbuch) Goodey the need arose for a rapid yet accurate method for determining the number of larvae in a suspension. Conventional counting methods depend for their accuracy on the precision with which very small volumes of the suspension can be measured, the number of larvae in this volume estimated, and the minimizing of sampling errors. Korsten *et al.* (1953) have described a colorimetric technique for determining quantitatively the concentration of the stem eelworm *Ditylenchus dipsaci* (Kühn) Filipjev. This method has greatly reduced the laborious task of counting, but in the construction of the standard transformation curve used in the estimation, dilution counting is employed and hence the considerable errors associated with dilution counts tend to be perpetuated in colorimetric estimations using this standard curve.

A counting method is outlined employing a modification of Peter's counting slide (Goodey, 1957), which it is suggested allows the construction of a transformation curve which can be used to estimate rapidly and more accurately the number of larvae in a suspension whose turbidity has been measured.

Experimental.

Ten 10 ml. suspensions of the larvae in a 0·5% solution of pure carboxymethylcellulose (c.m.c.) were prepared, the eelworm concentrations of which were representative of the range over which the curve was to be used for estimating larval numbers. The logarithm of the light absorption due to the turbidity of the suspension was measured for each suspension using a Hilger "Spekker" absorptiometer fitted with H508 filters. Each reading was standardized against a solution of the suspending fluid.

The number of larvae in each suspension whose light absorption has been measured must be determined by a direct counting method. A simply constructed modification of Peter's counting slide (Goodey, 1951) was introduced, to facilitate direct counting

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and to minimize the error associated with the count. The counting slide consists essentially of three parts:

(1) A microscope slide onto which two fine lines have been etched 12·5 mm. apart. (If concentrated suspensions are being counted, an additional centre line can be drawn.)

(2) Two traverse strips 10 mm. wide cut from a slide uniformly 1 mm. thick cemented across the first slide, one at each end.

(3) A third slide is cut longitudinally 14 mm. wide and cemented to the cross-pieces made by the portions of the second slide in a position as indicated in Figure 1.

The stabilized suspension containing the nematodes was introduced between the two slides in sufficient quantity to fill the volume between the two slides completely. The number of larvae enclosed by the etched lines in strips selected at random were

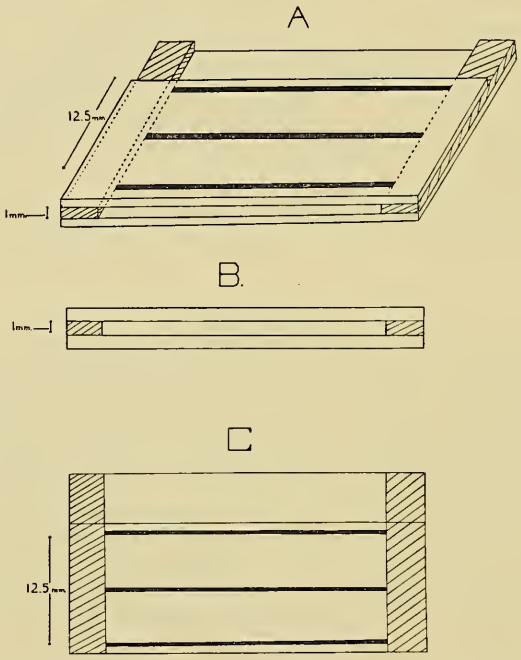

Fig. 1.—Counting slide. A, General view; B, Side view; C, Plan view. For description see text.

counted using a tally counter as the slide was moved across the low power field of the microscope by means of a mechanical stage. By proper adjustment of the viscosity of the c.m.c. suspending medium all the larvae can be brought into the same optical plane, thereby improving the rapidity and ease of counting.

From each standardizing suspension five slides were prepared and five strips counted at random on each slide. The mean number of larvae per strip was contained in a volume of fluid which equalled (*the distance between the etched lines*) × (*the depth of the cell*) × (*the diameter of the microscope field*). Knowing the original volume of the suspension, the number of larvae in the suspension can be calculated.

Using the logarithm of absorption and the number of larvae in each standardizing suspension a standard transformation curve was constructed as shown in Figure 2 and used subsequently to predict the number of larvae in a suspension whose logarithm of absorption only had been measured.

An implicit assumption of the method outlined is that the larvae are uniformly distributed throughout the suspension whose absorption is measured. To judge the effectiveness of c.m.c. in stabilizing the larvae suspension, the number of larvae in

five strips were counted at random on each of five slides. One set of slides was prepared from a suspension where the suspending fluid was 0·5% c.m.c. and the other set where the suspending fluid was distilled water. If the larvae are uniformly distributed then the slide counts will have a Poisson distribution and the index of dispersion will be distributed as a χ^2 (Fisher, 1948). The indices of dispersion have

TABLE 1.

A Comparison between Direct Slide Counting and Turbidity Measurement Methods for Estimating the Number of A. agrostis *Larvae in a Suspension.*

Logarithm of Absorption.	Mean Number of Larvae per 16·875 mm.³* of Suspension Determined by		Percentage Error.
	Direct Slide Counting.†	Turbidity Measurement.	
0·045	2·60	2·69	3·3
0·095	5·35	5·51	2·9
0·215	13·45	13·93	3·5

* Volume of fluid per strip counted = $1 \times 12{\cdot}5 \times 1{\cdot}35 = 16{\cdot}875$ mm.³

† Mean number of larvae per strip in 25 strips counted.

been calculated from the larvae counts per slide and with water as the suspending medium was 39·358, $P(\chi^2_4) > 39{\cdot}358$ is less than 0·01 and with c.m.c. as the suspending medium was 3·218, $P(\chi^2_4) > 3{\cdot}218$ falls between 0·5 and 0·7.

These results indicate that water was an unsatisfactory suspending medium. Also there was the suggestion that the c.m.c. suspension gave counts conforming to a Poisson distribution. To establish c.m.c. as a satisfactory medium it would be necessary

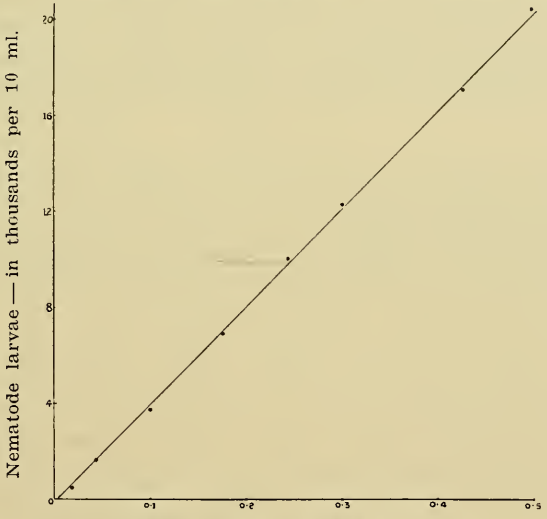

Fig. 2.—Standard transformation curve, showing the relationship between the number of nematode larvae per 10 ml. of suspension and the logarithm of the light absorption. Regression equation: Y = −168·3 + 39104X. Where Y = the estimated number of larvae per 10 ml. of suspension and X = measured logarithm of absorption of the suspension. Correlation coefficient (r) = 0·96 (significant 0·1% level).

to have a large number of observations of five sets of larvae counts, to compute Fisher's index of dispersion from these data and then to match the distribution of these values with that of the χ^2_4 distribution.

DISCUSSION.

The evidence presented confirms the findings of Korsten *et al.* (1953) that absorptiometric methods can be used to estimate reliably the number of nematode

larvae in a suspension. Figure 2 shows the amount of light absorbed by a suspension is linearly related to the number of larvae of *A. agrostis* in the suspension. Such a relationship allows the construction of a standard transformation curve which can be used to predict the number of larvae in a suspension whose turbidity has been measured. The reliability of this technique is shown by the results in Table 1.

In the design of the technique an attempt has been made to minimize counting errors. Percentage errors as small as those given in Table 1 are possible for a number of reasons:

(1) The use of c.m.c. as the suspending fluid reduces variability in the distribution of larvae in the suspension and hence the light absorption which is measured in a part of the suspension is representative of the whole suspension.

(2) When the counting slide is used large samples each of approximately 2 ml. are taken randomly from the suspension. The large sample taken from a stabilized larval suspension reduces sampling errors.

(3) The accuracy with which the direct count is made using the counting slide provides a sound basis for estimating the accuracy of the indirect method as has been done in Table 1.

Satisfactory techniques have been described for making total counts of mixed nematode populations (Goodey, 1957) and which in addition enable the frequency of the component genera in a population to be determined. Where a reliable estimate of the total number of nematodes present is required, indirect turbidimetric counting can only usefully be employed where a large and uniform nematode population is involved.

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